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Synthesis of Molecularly Imprinted Membrane Glucose for Selective Membrane Transport

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Abstract

Molecularly Imprinted Membrane (MIM) was synthesized using polyeugenoxy acetic acid as the functional polymer, polyethylene glycol as the crosslinker agent, and polysulfone as a base membrane which was applied as a selective glucose membrane transport, and the immersion time expected to determine the transport capability of the membrane. This study aimed to determine the selectivity and transport properties of the MIM and NIM membranes. NIM was used as a control for MIM to research the selectivity test. In comparison, MIM has a template, while NIM is without a template. In this study, eugenol derivatives were synthesized through a polymerization reaction using a BF3-diethylether catalyst polymerized for 16 hours to produce polyeugenoxy acetic acid (PA). The PA was contacted with 7500 ppm glucose. PA-glucose produced an imprinted membrane, while PA produced a non-imprinted membrane. The membrane thickness was measured with a micrometer, resulting in a measurement range of 0.08–0.10 mm. The best transport result was achieved at the membrane passage of 24 hours of immersion time because the effect of membrane immersion time can increase the porosity, hydrophilicity, and membrane's transport ability. Transport with MIM membrane shows better and more selective results than NIM. This confirms the existence of a glucose template on the MIM membrane, which causes the MIM membrane to recognize glucose and transport glucose better than fructose. This study's advantages include learning how immersion time affects membrane production and determining how well MIM and NIM membranes transport and select glucose and fructose. Furthermore, membrane characterizations were done using FTIR to identify functional groups, SEM-EDX to analyze the shape of the membrane, and a UV-Vis spectrophotometer to analyze the membrane's selectivity and transport capabilities.

1. Introduction

Diabetes mellitus is a group of metabolic disorders that affect humans because the body cannot regulate blood sugar levels due to the ineffective production of insulin in the pancreas [1]. Treatment for this disease is to apply a glucose diet, which can be obtained by providing glucose-free sugar, selectively adsorbing glucose from fructose, and leaving fructose safe for people with diabetes. Molecularly Imprinted Polymer (MIP) molecular adsorption provides a selective glucose absorption technology based on functional monomers and linkers around template molecules [2].

From a previous study, Djunaidi and Astuti [3] have developed glucose MIP with the result that MIP has better glucose selectivity than NIP and does not absorb fructose. Djunaidi and Wenten [4] also researched the synthesis of selective eugenol-based membranes for hemodialysis. The study revealed that MIM urea transported urea more effectively than NIM. The more thin the membrane, the more efficient the urea transport process. Creatinine can also be transported by MIM urea, but not vitamin B-12. Adsorption using MIP yielded higher and more selective results than NIP, indicating that MIP's glucose template enabled it to identify glucose molecules more effectively than NIP. Similarly, the urea template of the MIM

membrane allows it to recognize urea molecules and makes it more efficient at transporting urea than the NIM membrane.

In this study, Molecularly Imprinted Membrane (MIM) and Non-imprinted Membrane (NIM) membranes were synthesized using a phase inversion technique which is performed by eliminating the solvent from a liquid-polymer solution and creating a porous, solid membrane in its place using polyeugenoxy acetic acid (PA) as a functional polymer with a polysulfone base membrane (PSf) and polyethylene glycol (PEG) as crosslinker. Polyeugenoxy acetic acid is a derivative of eugenol, which is one of the natural native products in Indonesia that has many functions [5]. Eugenol is a non-polar organic compound that can be used as a starting material for synthesizing compounds due to the attachment of three functional groups: hydroxyl, allyl, and methoxy groups [6]. In MIM synthesis, polyeugenoxy acetic acid is first contacted with glucose, which is then released from the MIM membrane in the membrane immersion process. MIM selectivity mechanism involves –OH group and glycidyl ether group. Novelty in this research uses polyeugenoxy acetate for function polymer to imprint glucose and polysulfone for the base membrane to transport glucose.

2. Methodology

2.1. Materials

Eugenol, $BF_3O(C_2H_5)_2$, NMP (1-Methyl-2-Pyrolidone), AIBN (2,2', Azobis (2-methyl propionitrile)), polysulfone, 3,5 Dinitrosalisylic acid (DNS), and polyethylene glycol 6000 were purchased from Sigma-Aldrich (Jakarta, Indonesia). Chloroform, methanol, Na_2SO_4 anhydrous, HCl, NaOH, diethyl ether, Cl_2CH_2COOH, $NaHCO_3$, D-glucose, D-fructose, K-Na Tartrate ($KNaC_4H_4O_6$), Na_2HPO_4, NaH_2PO_4 were purchased from Merck (Jakarta, Indonesia) and double distilled water was purchased from Bratachem (Semarang, Indonesia). All chemicals were of analytical grade, excluding double distilled water.

2.2. Polymerization of Eugenol

A 5.8 g of eugenol was added to a three-necked flask, followed by BF3-diethyl ether. BF3-diethyl ether was added 1 mL dose over 4 hours, each dose being 0.25 mL once an hour, while the mixture was agitated with a stirrer for 4 hours. After 16 hours of polymerization, 1 mL of methanol was added to end the process. The polymerization result was dissolved in 100 mL of chloroform and neutralized to neutral pH by adding water. The water was then completely removed using anhydrous Na_2SO_4 and filtered. It was left for 24 hours until the chloroform solvent evaporated. The polyeugenol was ground to obtain a pink powder, and the results of polymer structures were analyzed using FTIR.

2.3. Synthesis of Polyeugenoxy Acetic Acid

A total of 5 g of polyeugenol was added to a double-neck flask, followed by 17.5 mL of 33% NaOH solution. The mixture was refluxed for 24 hours at 80–90°C under continuous stirring. Then, 12.5 mL of 50% chloroacetic acid solution was slowly added by a pipette while stirring was continued. The mixture was cooled and acidified with 6 M HCl until pH 1 was reached. It was extracted three times with diethyl ether, each with an extraction volume of 50 mL. The ether extracts were combined and extracted three times with Na_2CO_3 5% w/v with a volume of 30 mL for each extraction. The aqueous layer was acidified with 6 M HCl until pH 1 was reached. Afterward, it was filtered, dried, and the result was weighed. The obtained results were analyzed using FTIR.

2.4. Synthesis of Molecularly Imprinted Membrane (MIM)

The synthesis was started by dissolving 3.4790 g of polysulfone in 12 mL of NMP (1-methyl-2-pyrrolidone) until completely dissolved, followed by adding 0.8333 g PEG as a crosslinking agent, 0.8333 g PA-Glucose as a functional polymer, and 0.249 mL of AIBN. The mixture was refluxed for about 10 hours at 90–100°C. After allowing the synthesis results to stand for 24 hours, the membrane mixture was poured onto the glass surface, adjusted to the film thickness from 0.08 mm to 0.10 mm, and immediately immersed in double distilled water with different times of 5, 12, and 24 hours. Membrane immersion was used to release the template from MIM cavities to investigate the impact of different immersion times on the printed pores. After immersion, the distilled water was examined using the DNS method to determine how much glucose had been released.

2.5. Synthesis of Non-Imprinted Membrane (NIM)

The synthesis of Non-Imprinted Membrane (NIM) was performed similarly to the synthesis of MIM. However, the difference was that NIM used PA instead of PA-Glucose. The samples of membranes are identified in Table 1.

Table 1. Sample identification

Sample code	Identification
MIM 5	MIM with 5 hours of immersion time
MIM 12	MIM with 12 hours of immersion time
MIM 24	MIM with 24 hours of immersion time
NIM 5	NIM with 5 hours of immersion time
NIM 12	NIM with 12 hours of immersion time
NIM 24	NIM with 24 hours of immersion time

2.6. Characteristics of MIM Glucose and NIM

MIM glucose and NIM were characterized by measuring membrane hydrophilicity, porosity, and water uptake, and analyzed using FTIR and SEM-EDX.

2.7. Glucose Transport

Glucose membrane transport was performed using the diffusion cell device, wherein the feed phase (feed, glucose) was 300 mg/L glucose, and in the receiving

phase was buffer phosphate (pH 7.4); each chemical was 50 mL, stirred for 24 hours, and sampled every 0, 8, 16, and 24 hours.

2.8. MIM Glucose Performance Test

Variations in immersion time. The performance of the membrane variations was tested to transport 300 ppm glucose.

Variations in glucose concentration. The glucose concentration in the feed phase (feed/glucose) was varied by 100, 200, and 300 ppm. Glucose transport was performed at MIM 24.

2.9. Fructose Transport

Fructose membrane transport was performed using the diffusion cell device, wherein the feed phase (feed, fructose) was 100 mg/L fructose, and in the receiving phase was buffer phosphate (pH 7.4); each chemical was 50 mL, stirred for 24 hours, and sampled every 0, 8, 16, and 24 hours.

3. Results and Discussion

3.1. Polyeugenoxy Acetic Acid Synthesis

Polyeugenol, a compound derived from eugenol, was synthesized successfully with a 92.24% yield. The percentage of polyeugenol was measured using formula (1).

$$\% \text{ Yield} = \frac{\text{Eugenol weight}}{\text{Polyeugenol weight}} \times 100\% \quad (1)$$

Polyeugenol was analyzed using gel permeation chromatography (GPC) to determine the number of *n* eugenol molecules in the polyeugenol chain. The results show that the average Molecular Mass (Mr) of polyeugenol is 1876, which is about 11 times higher than the monomer (eugenol has a Mr of 164) [7]. Polyeugenoxy acetic acid was also synthesized successfully with a yield of 85.4%. The structure of polyeugenol and polyeugenol acetic acid are shown in Figures 1 and 2.

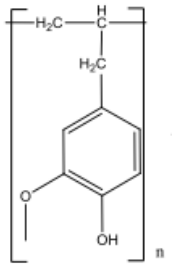

Figure 1. The structure of polyeugenol

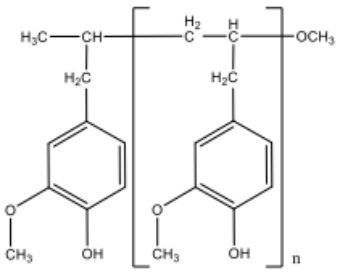

Figure 2. The structure of polyeugenoxy acetic acid

Then, the result of polymer structures was analyzed using FTIR to determine the polymers' functional group and vibration type. The FTIR spectra of eugenol and its derivatives are shown in Figure 3.

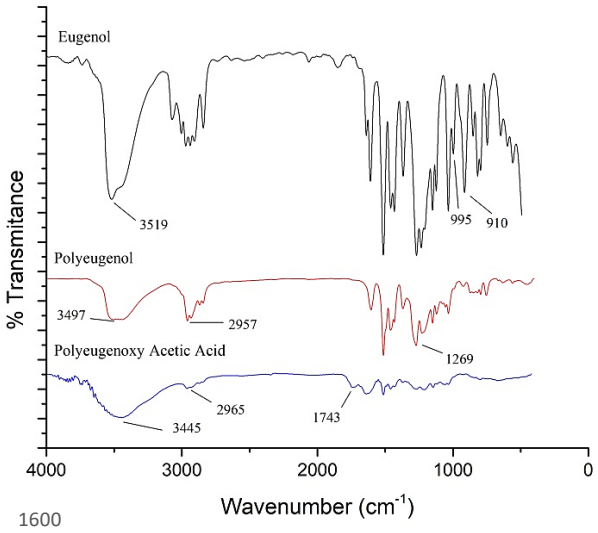

Figure 3. FTIR spectra of eugenol and its derivatives

It shows that before the polymerization, a vinyl group was indicated by the presence of absorption bands at 995 cm^{-1} and 910 cm^{-1} in eugenol. However, in polyeugenol, there is no absorption in these areas. This happens because the vinyl groups are modified to bond with other eugenols to form polyeugenol. There are absorption bands at 2958.48 cm^{-1}, and reinforced absorption band 1458.5 cm^{-1} in eugenol shows the characteristic C-H stretching of the methyl group, absorption band 3497 cm^{-1} in polyeugenol and 3445 cm^{-1} in polyeugenoxy acetic acid, indicating the presence of a hydroxyl group (OH). Methylene group ($-CH_2-$) in the absorption bands 2957 cm^{-1} and 2965 cm^{-1}. Acid C-O bonds are identified at the 1269 cm^{-1} absorption band, while the alcohol carbonyl group (1600 cm^{-1}) changed to acid carbonyl groups at the 1743 cm^{-1} absorption band. From these data, it can be concluded that polyeugenol and polyeugenoxy acetic acid have been successfully synthesized.

3.2. Synthesis of MIM Glucose and NIM Results

Polyeugenoxy acetic acid (PA), a polymer not in contact with glucose, is employed to synthesize NIM. The membrane will be able to identify the target molecule (glucose) when PA-Glucose is used to synthesize MIM. The approximate reaction between PEG, polysulfone, and PA-Glucose is shown in Figure 4.

From the estimation reaction, it can be seen that PEG has two OH groups at each end. Adding PEG as a crosslinker improves the structural morphology and properties of the membrane [8]. The OH group at one end is bonded to the polysulfone on the benzene near the -O- and O=S=O groups. This is because the -O- group is the ortho-director supported by the O=S=O group as the meta-director [9]. The remaining -OH end of PEG is attached to PA-Glucose as its functional polymer. The reaction mechanism that occurs in this synthesis is also based on the research conducted by Fan and Wang [8] that

the double bond in methacrylic acid (MAA) can be crosslinked with glycol methacrylate (GDMA) by the addition of the AIBN as the initiator to crosslink eugenol which may not have been crosslinked. In addition, polysulfone can also be crosslinked with the addition of PEG. FTIR analysis was performed to determine the effect of immersion time on membrane functional groups.

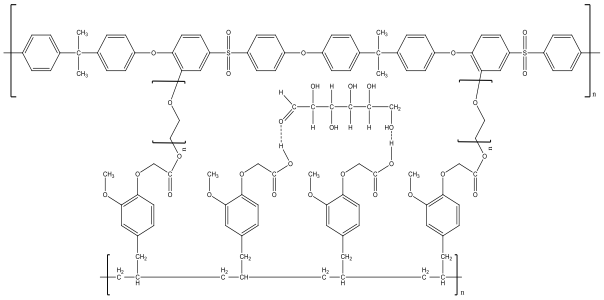

Figure 4. Estimation of crosslink bonding in MIM glucose

In a previous study, Djunaidi *et al.* [10] also conducted research similarly but for applying urea-selective membrane transport. The existence of crosslinks between the benzene groups on Psf can be seen from the decreasing peak of the O-H group at 3450–3460 cm^{-1} along with the longer heating time and evidenced by the increasing C-O spectra of the C-benzene and O bonds of the PEG molecule. Because fewer molecules are being crosslinked when the heating process is insufficient, the intensity of the OH absorption peak will be high, and the C-O absorption will be low. The heating process carried out in membrane synthesis was for 10 hours so that the resulting membranes could be well crosslinked. The reactions on NIM membranes are similar to those on MIMs, with the only difference being that there is no glucose template on the membrane. The crosslinking reaction in the membrane manufacturing process can also lock the glucose ion template to the PA polymer to form an appropriate imprinted mold [10]. The results of the FTIR analysis on the variation of the MIM and NIM membranes are shown in Figure 5. The imprinted membrane is produced by varying the immersion period for each sample, with different times of 5, 12, and 24 hours. The imprinted membrane was identified as MIM 5, MIM 12, MIM 24, NIM 5, NIM 12, and NIM 24 for non-imprinted membranes.

Based on the FTIR spectra in Figure 5, PEG crosslinks prevent the -OH group's absorption peak at the wavenumber 3200–3650 cm^{-1} from appearing. This is because the -OH group in PEG has bonded with polyeugenoxy acetic acid and polysulfone, as shown in Figure 5. S=O groups derived from polysulfone are present at the absorption band 1242–1246 cm^{-1}, while C–O groups are at 1144–1148 cm^{-1}. This result is supported by Song *et al.* [11], who identified the presence of the S=O group in the modified polysulfone at 1295 cm^{-1} absorption band and indicated the presence of a (C-O) group at 1125–1148 cm^{-1} due to the reaction between polysulfone and PEG.

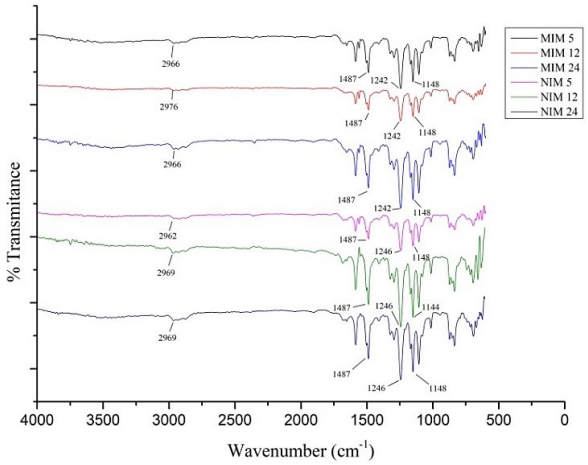

Figure 5. FTIR spectra of MIM and NIM

The MIM and NIM membranes were characterized using SEM-EDX to determine their pore size, morphology, membrane surface area, and composition of the elements. The results of SEM analysis were then processed using ImageJ software to sharpen the resulting pore images. Figures 6 and 7 show the surface morphologies and cross-section of MIM and NIM membranes at 5000× magnification.

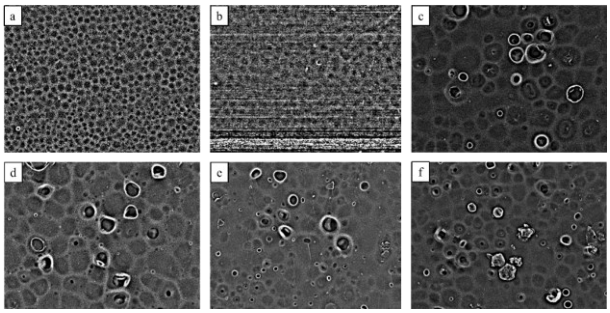

Figure 6. Membrane morphology images of (a) MIM 5, (b) MIM 12, (c) MIM 24, (d) NIM 5, (e) NIM 12, (f) NIM 24

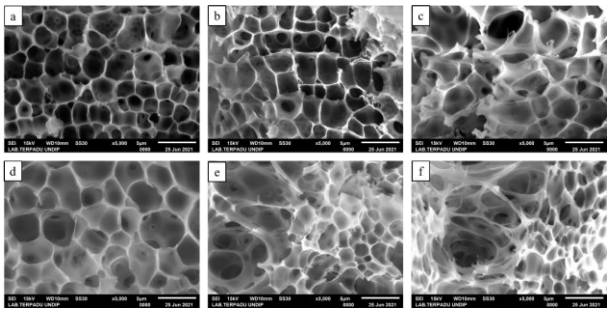

Figure 7. Cross-section SEM images of (a) MIM 5, (b) MIM 12, (c) MIM 24, (d) NIM 5, (e) NIM 12, (f) NIM 24

Based on Figures 6 and 7, SEM analysis of variations in MIM membranes reveals that MIM 24 has a larger average pore size than MIM 5 and MIM 12. The SEM study of the membrane surface revealed that the membrane immersion time affects the membrane pores. The longer the immersion time of the membrane, the larger the pore size of the membrane. This is possible because the pore cavity enlarges as the amount of glucose released from the membrane increases. NIM membranes with different variations have varying and uneven pore sizes for NIM analysis. NIM has a higher average pore size compared to

MIM when the membrane variation is considered. The average pore sizes of MIM and NIM variations are shown in Table 2.

Table 2. The average pore size of membranes

Membran type	Pore size (µm)
MIM 5	0.0894
MIM 12	0.0786
MIM 24	1.625
NIM 5	1.831
NIM 12	1.249
NIM 24	1.655

Those results in Table 2 are in accordance with the research conducted by Scorrano *et al.* [12], where the pore sizes of the MIM membranes were smaller or decreased compared to the membrane that had not been imprinted (NIM). The EDX results are shown in Table 3 for the composition of the elements in MIM and NIM. It can be seen that the amount of elemental O mass is less than that of MIM, which suggests that the NIM membrane variant does not contain any glucose molecules. In contrast, the S element is a functional group generated from polysulfone.

Table 3. EDX analysis results of the elements in MIM and NIM

Element	MIM5 (%)	MIM 12 (%)	MIM 24 (%)	NIM 5 (%)	NIM 12 (%)	NIM 24 (%)
C	76.45	77.89	77.55	77.05	77.52	77.23
O	19.40	18.19	17.55	16.41	17.78	17.90
S	4.60	3.91	5.46	4.39	4.51	4.63

3.2.1. Membrane Hydrophilicity

Hydrophilicity was analyzed using the DSA method (Drop Shape Analysis) by measuring the contact angle of the membrane and observing the sessile drop. The membrane is hydrophilic if the contact angle value is less than 90° and hydrophobic if it is more than 90° [13]. The results of the membrane hydrophilicity test can be seen in Figure 8 and Table 4.

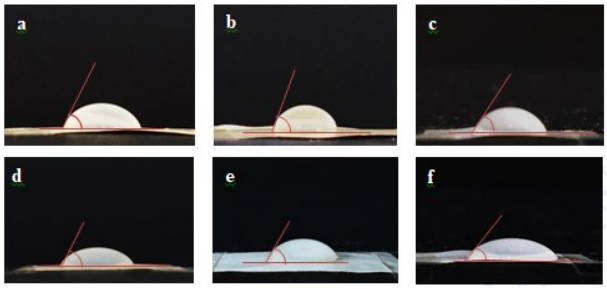

Figure 8. Membrane hydrophilicity of (a) NIM 5, (b) NIM 12, (c) NIM 24, (d) MIM 5, (e) MIM 12, (f) MIM 24

Glucose generally contains many hydrophilic hydroxyl groups (-OH). Therefore, the addition of glucose molecules causes the membrane surface to become more hydrophilic, thereby increasing membrane permeability [14, 15]. Figure 6 and Table 4 demonstrate that the addition of glucose molecules and membrane immersion time can increase membrane hydrophilicity. This is supported by a decrease in the contact angle value of the membrane from 69.68° to 43.85°.

Table 4. The contact angle of different membrane types

Membrane type	Contact angle
NIM 5	69.68°
NIM 12	60.62°
NIM 24	53.26°
MIM 5	48.37°
MIM 12	48.09°
MIM 24	43.85°

3.2.2. Porosity Test

Porosity tests, usually performed on the water, are used to determine the amount of substance that can be adsorbed by a membrane [16]. The membrane porosity test used the weighing method, which involved determining the ratio of pore volume to the total volume of the membrane [17]. The results of the porosity test can be seen in Figure 9.

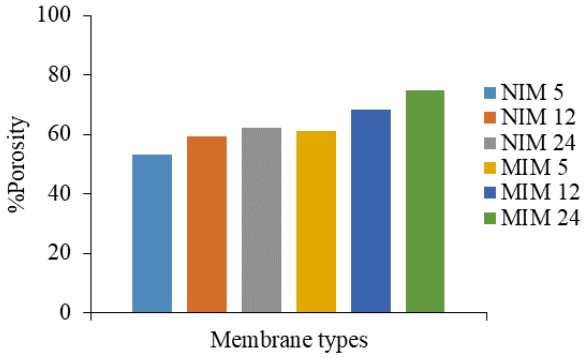

Figure 9. Graph of porosity test

From Figure 9, the maximum porosity value was obtained by MIM 24 with a value of 74.93%. The glucose molecule is responsible for these results because the presence of the -OH group causes the membrane to become more hydrophilic, resulting in a phase inversion process in the solvent which can increase the porosity of the membrane [18]. The -OH groups also increase the regularity of the polymer structure at a given distance, resulting in more uniform and regular cavities. The solvent will be encouraged to fill the vacant space at the membrane interface when the membrane expands due to the increased mobility of the polymer chain.

3.2.3. Water Uptake Test

The water uptake test aims to measure the ability of a membrane to absorb water. This test was carried out using the weighing method. The membrane weight before and after immersion was used to determine the membrane's percent water absorption. The results can be seen in Figure 10.

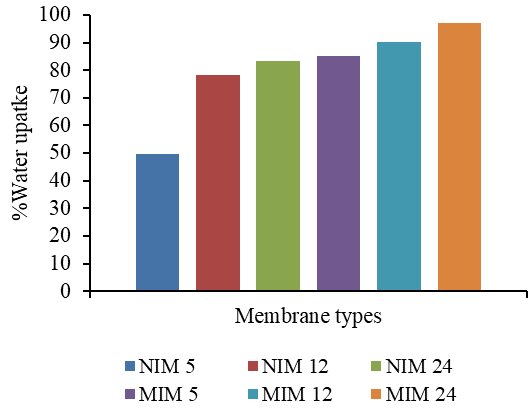

Figure 10. Graph of water uptake test

From Figure 10, MIM 24 has the largest %water absorption at 97.05%. The addition of glucose, which has a hydrophilic -OH group and increases the membrane's hydrophilicity, causes an increase in the percentage of water absorption in the membrane. The increased water absorption value is directly proportional to the porosity value of the test results because membranes with small pores make it challenging for water to pass through. Water can easily flow through a membrane if its pores are large [19]. MIM 24, MIM 12, MIM 5, NIM 24, NIM 12, and NIM 5 are listed in order of the percentage of water uptake from the highest to the lowest.

3.2.4. Glucose Transport with Membrane Immersion Time Variations

The percentage of transport was determined by making absorbance graphs for membranes with 5, 12, and 24 hours of immersion. Equation (2) was used to calculate the percentage of transportation.

$$\%Transport = 100 - feed\ phase \quad (2)$$

The graph in Figure 11 shows that the MIM membrane produced the optimum glucose transport with an immersion time of 24 hours, where the glucose concentration in the receiving phase increased by 49.15% within 24 hours. From the receiving phase, the percentages of glucose delivered to MIM 5, MIM 12, NIM 5, NIM 12, and NIM 24 were 11.62%, 23.94%, 5.71%, 4.44%, and 19.12%, respectively. The results of transport using the MIM membrane demonstrate greater transport performance than the NIM membrane, showing the successful formation of a glucose template on the MIM membrane and the greater ability of the MIM membrane to recognize and transport glucose over the NIM membrane. As a result, it is clear that the duration of the immersion time impacts the membrane's capacity for transport and that 24 hours is the optimum duration.

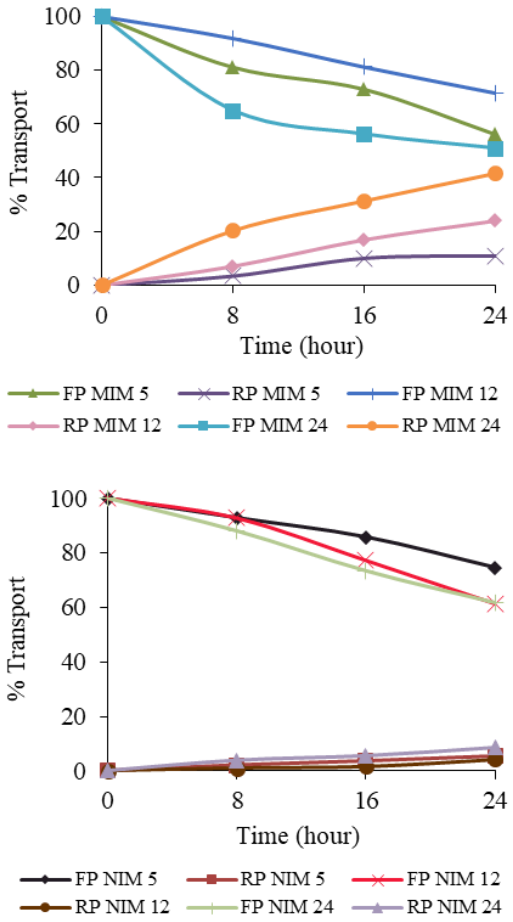

Figure 11. Immersion time variations for glucose transport between MIM and NIM

3.2.5. Transport of Glucose in Different Concentrations

In this transport test, the performance of the membrane with increased glucose concentration was evaluated over 24 hours with transport at different glucose concentrations of 100, 200, and 300 ppm [20]. A membrane with a 24-hour immersion is used as the transport membrane. Transport to the NIM membrane was also performed as a comparison to the MIM membrane. Compared to 100 ppm and 200 ppm values, the results showed that the 24-hour MIM membrane performed best at a glucose concentration of 300 ppm. Figure 12 shows that glucose concentration also determines the rate of glucose transport through the membrane. The higher the glucose concentration, the higher the glucose concentration is transported. This is because transport occurs when there is a concentration difference between the buffer phase /receiving phase (RP) and the glucose phase/feed phase (FP).

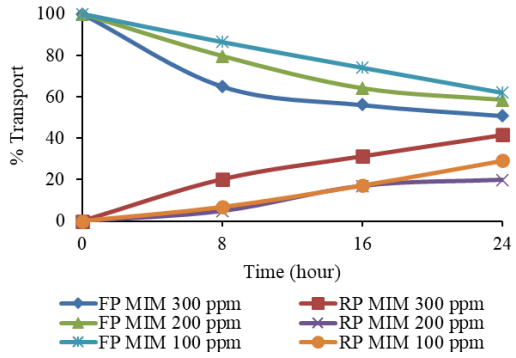

Figure 12. Comparison of glucose transport in the variation of glucose concentrations

3.2.6. Transport of Fructose

The fructose transport test used fructose with concentration of 100 ppm. It can be seen in Figure 13 that MIM glucose can also transport fructose, but to a lesser extent than glucose. These transport results are used to determine membrane selectivity.

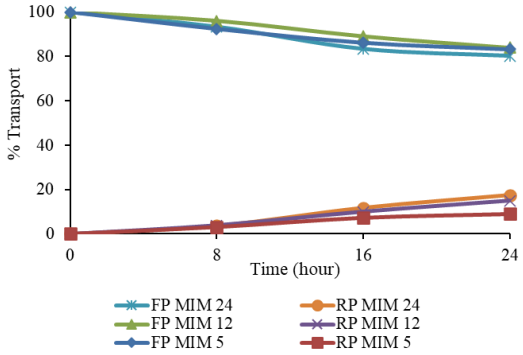

Figure 13. Fructose transport by MIM glucose

3.2.7. Selectivity Test Results

A glucose selectivity test on the MIM and NIM membranes was performed by comparing the results of glucose transport with data on the fructose transport results using various MIM and NIM membranes.

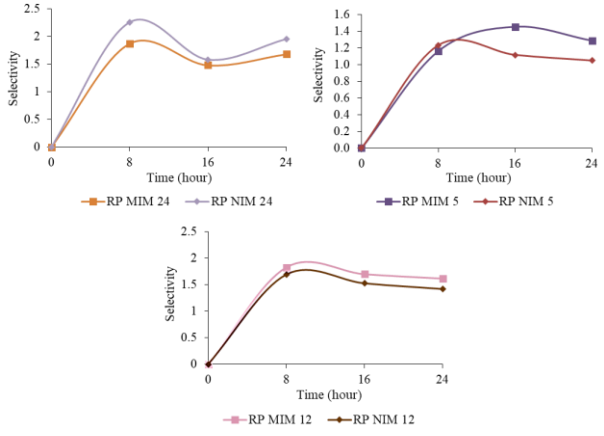

Figure 14. Graph of membrane selectivity based on receiving phase

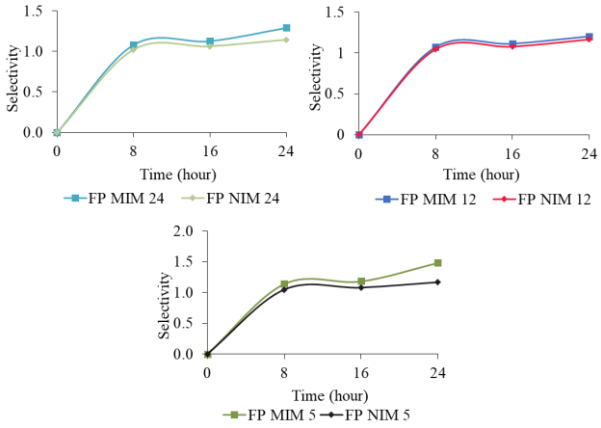

Figure 15. Graph of membrane selectivity based on feed phase

As shown in Figures 14 and 15, the MIM membranes show a higher selectivity value than NIM membranes. It means that the MIM membrane can transport glucose better than fructose. This indicates the presence of a glucose template on the MIM membrane, which makes the MIM membrane more selective for glucose and unable to transport fructose [7]. Although MIM 24 has the bigger porosity, the selectivity test membrane with an immersion time of 12 hours has better selectivity than 24 hours. It happens because of the membrane permeability; the higher the flux of the membrane, will decrease the membrane selectivity. The selectivity results in the feed phase are shown in Table 5 and Figure 16.

Table 5. Selectivity test results in the feed phase

Time (hour)	NIM 24	MIM 24	NIM 12	MIM 12	NIM 5	MIM 5
8	1.02	1.08	1.05	1.05	1.05	1.14
16	1.06	1.13	1.08	1.10	1.09	1.18
24	1.14	1.29	1.17	1.18	1.23	1.48

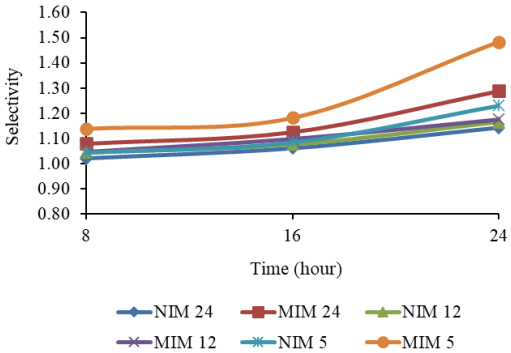

Figure 16. Selectivity results in the feed phase

4. Conclusion

This study's findings support the successful synthesis of polyeugenol and polyeugenoxy acetic acid, two eugenol derivatives. The molecularly imprinted and non-imprinted membranes, with an average thickness of roughly 0.08–0.10 mm, have been successfully synthesized. Increases in membrane porosity, hydrophilicity, and transport capacity may result from longer immersion times. Glucose transport using MIM

showed satisfactory results as it is greater than NIM. This is due to the MIM glucose template. The molecularly imprinted membrane is more selective for glucose and fructose solutions than non-imprinted membranes.

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